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Original

De Novo Synthesis of Veratryl Alcohol by *Coriolus versicolor**

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Abstract——Veratryl alcohol was found in ligninolytic culture of *Coriolus versicolor*. The structure of veratryl alcohol synthesized *de novo* was comfirmed in comparison with ^{1}H-NMR spectra of the authentic dimethoxybenzyl alcohols (veratryl alcohol and its isomers).

1. Introduction

Lignin biodegradation has been greatly elucidated in recent years[1~5]. Ligninolytic enzyme (lignin peroxidase, ligninase) was purified from the culture filtrate of *Phanerochaete chrysosporium*[6~9] and the reaction mechanism of lignin peroxidase *via* aryl cation radical was proposed[10,11]. This enzyme activity was enhanced by the addition of veratryl alcohol, a secondary metabolite of *P. chrysosporium*[12].

We previously reported the degradation of non-phenolic β-*O*-4 lignin substructure model compounds in ligninolytic culture of *Coriolus versicolor* and suggested that a similar lignin peroxidase is excreted by *C. versicolor*[13~15]. In the present paper we report *de novo* synthesis of veratryl alcohol by *C. versicolor* and discuss the role of veratryl alcohol in lignin biodegradation.

2. Materials and Methods

2.1 Culture Conditions and Extraction

Coriolus versicolor Ps4a was maintained on 2% malt agar slants. Experimental culture (20 ml in 300 ml-Erlenmeyer flasks) were inoculated with a small mycelial mat from the slant and grown without agitation at 30°C. The culture medium was prepared as described previously[13].

The 7-day-old cultures (28 cultures) were flushed with sterile oxygen and incubated under the same conditions for 3 days. The whole cultures were combined, acidified with 1N HCl to pH 2 and extracted with 1 liter of ethyl acetate. The

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organic layer was washed with saturated NaCl solution, dried over anhydrous Na_2SO_4 and concentrated to dryness.

2.2 Syntheses of Authentic Compounds

2,3-Dimethoxybenzyl alcohol was prepared from 2-hydroxy-3-methoxybenzaldehyde (*o*-vanillin, Nakarai Chemicals Ltd.) *via* the following two steps; (i) methyl iodide/K_2CO_3 in DMF at room temperature, and (ii) $NaBH_4$ in methanol at 0°C.

^{1}H-NMR ($CDCl_3$) δ (ppm); 3.87 (3H, s, $-OCH_3$), 3.89 (3H, s, $-OCH_3$), 4.70 (2H, s, $-CH_2-$), 6.82–7.12 (3H, m, aromatic-H).

2,4-Dimethoxybenzyl alcohol was prepared from 2,4-dihydroxybenzaldehyde (β-resorcylaldehyde, Nakarai Chemicals Ltd.) *via* the following two steps; (i) methyl iodide/K_2CO_3 in DMF at room temperature, and (ii) $NaBH_4$ in methanol at 0°C.

^{1}H-NMR ($CDCl_3$) δ (ppm); 3.80 (3H, s, $-OCH_3$), 3.83 (3H, s, $-OCH_3$), 4.60 (2H, s, $-CH_2-$), 6.40–6.46 (2H, m, aromatic-$H_{3,5}$), 7.16 (1H, d, J=9.0, aromatic-H_6).

2,5-Dimethoxybenzyl alcohol was prepared from 2,5-dimethoxybenzaldehyde (Nakarai Chemicals Ltd.) by reduction with $NaBH_4$ in methanol at 0°C.

^{1}H-NMR ($CDCl_3$) δ (ppm); 3.77 (3H, s, $-OCH_3$), 3.81 (3H, s, $-OCH_3$), 4.65 (2H, s, $-CH_2-$), 6.76–6.90 (3H, m, aromatic-H).

3,4-Dimethoxybenzyl alcohol (veratryl alcohol) was commercially available (Tokyo Chemical Industry Co., Ltd.).

^{1}H-NMR ($CDCl_3$) δ (ppm); 3.87 (3H, s, $-OCH_3$), 3.88 (3H, s, $-OCH_3$), 4.61 (2H, s, $-CH_2-$), 6.78–6.94 (3H, m, aromatic-H).

2.3 Instrument

^{1}H-NMR spectra were obtained with a Varian XL-200 FT-NMR spectrometer (200 MHz) using tetramethylsilane as an internal standard. Chemical shifts and coupling constants are given in δ values (ppm) and Hz, respectively.

3. Results and Discussion

The extracts were submitted to TLC (Kiesel gel 60, F_{254}, Merck, developing solvent: CH_2Cl_2). Veratryl alcohol was isolated and its structure was identified by ^{1}H-NMR. The ^{1}H-NMR spectra of the metabolic veratryl alcohol and authentic compounds are shown in Fig. 1. Possibility of 3,5-dimethoxybenzyl alcohol is ruled out, because the protons of the two methoxyl groups of the metabolite have different chemical shifts in ^{1}H-NMR spectrum, while the chemical shifts of methoxyl groups of 3,5-dimethoxybenzyl alcohol are identical. From the ^{1}H-NMR spectra shown in Fig. 1, it is clear that the metabolic product (A) is veratryl alcohol (B) and not other isomers (C–E).

Russell *et al.*[16] found veratraldehyde in a culture of *C. versicolor*. *De novo* syn-

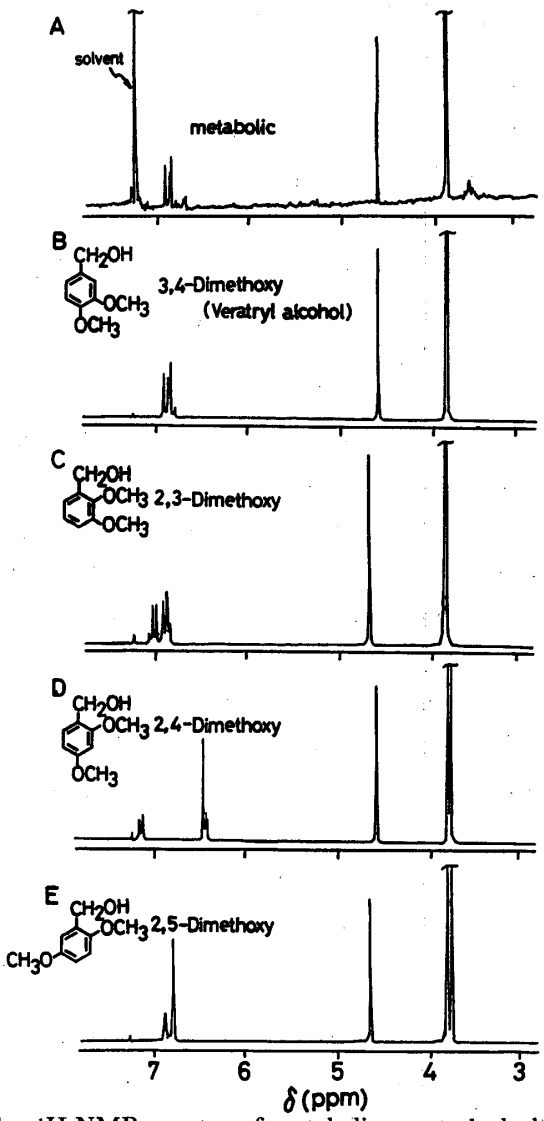

Fig. 1. ^{1}H-NMR spectra of metabolic veratryl alcohol (A) and authentic compounds (B–E).

thesis of veratryl alcohol by *P. chrysosporium* was reported previously by Lundquist and Kirk[17]. Afterwards many papers were published in relation to physiological and biochemical role of veratryl alcohol in lignin biodegradation by *P. chrysosporium*. Shimada *et al.*[18] reported the biosynthesis of veratryl alcohol in relation to lignin degradation by *P. chrysosporium*. It was demonstrated that addition of veratryl alcohol to the culture of *P. chrysosporium* increased the ligninolytic activity and the production of lignin peroxidase in the culture[12,19]. It was further reported that the oxidation of monomethoxylated aromatic monomers[20] and 2-keto-4-thiomethyl butylic acid (KTBA)[21] by lignin peroxidase of *P. chrysosporium* was enhanced by veratryl alcohol.

Our previous investigations[13~15] showed that the main degradation products of non-phenolic β-*O*-4 lignin substructure model compounds by *C. versicolor* were similar to those obtained by lignin peroxidase of *P. chrysosporium*[6~9,22,23], suggesting that a similar lignin peroxidase is excreted by *C. versicolor*. These results also suggest that veratryl alcohol enhances the ligninolytic activity and the production of the lignin peroxidase by *C. versicolor*.

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